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Ultrasound Imaging Velocimetry with interleaved images for improved pulsatile arterial flow measurements: a new correction method, experimental and *in vivo* validation

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Running title: Interleaved UIV for arterial blood flow

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Abstract

Blood velocity measurements are important in physiological science and clinical diagnosis. Doppler ultrasound is the most commonly used method but can only measure one velocity component. Ultrasound imaging velocimetry (UIV) is a promising technique capable of measuring two velocity components; however, there is a limit on the maximum velocity that can be measured with conventional hardware which results from the way images are acquired by sweeping the ultrasound beam across the field of view. Interleaved UIV is an extension of UIV in which two image frames are acquired concurrently, allowing the effective inter-frame separation time to be reduced and therefore increasing the maximum velocity that can be measured. The sweeping of the ultrasound beam across the image results in a systematic error which must be corrected: in this work we derived and implemented a new velocity correction method which accounts for acceleration of the scatterers. We then, for the first time, assessed the performance of interleaved UIV for measuring pulsatile arterial velocities by measuring flows in phantoms and *in vivo* and comparing the results with spectral Doppler ultrasound and transit-time flow probe data. The velocity and flow rate in the phantom agreed within 5-10 % of peak velocity, and 2-9% of peak flow, respectively and *in vivo* the velocity difference was 9 % of peak velocity. The maximum velocity measured was 1.8 m/s, the highest velocity reported with UIV. This will allow flows in diseased arteries to be investigated and so has the potential to increase diagnostic accuracy and enable new vascular research.

Key words: ultrasound, blood flow, atherosclerosis, haemodynamics, particle image velocimetry, echo-PIV

1. Introduction

Arterial blood velocity measurements are used as a diagnostic tool in diseases including atherosclerosis, coarctation and dissection [1-3]. Velocity measurements in the heart provide information about valve areas and regurgitation, cardiac shunts, and ventricular dysfunction [4-6]. Measurements of blood velocity are also an important tool in cardiovascular science and are used extensively in animal models of disease and development [7]. In addition, blood velocity measurements are useful in the development of medical devices such as artificial valves, stents and heart pumps [8].

Doppler ultrasound is currently the chief clinical method for measuring blood velocity [9]. However, an inherent limitation of the standard Doppler method is that only the velocity component parallel to the ultrasound beam is measured. If the angle between the direction of blood flow and the ultrasound beam is known, it is simple to convert the velocity component to velocity magnitude. In vascular ultrasound the blood is often assumed to flow parallel to the wall of the vessel. However, this is not always the case, and there are many situations where the blood direction is unknown, particularly in areas of non-cylindrical geometry or disease. Inaccurate angle and sample volume placement causes velocity errors up to 28 % [10]. Even when angle corrections can be performed, Doppler methods suffer from several sources of error [11]. As an example of the impact these errors have in the clinic, consider diagnosis of 70% carotid stenosis. Using the peak systolic velocity measured by Doppler 7% of stenoses are undiagnosed leaving patients at risk of transitory ischaemic attack or stroke, while 7% of the >5000 patients in the UK [12] undergoing carotid endarterectomies each year may be having unnecessary surgery [13].

Many methods have been proposed to overcome these limitations (reviewed by Taylor and Draney [14] and Evans [15, 16]). The most notable are vector Doppler and speckle tracking [17, 18]. Since 2000 a technique known as Ultrasound Imaging Velocimetry (UIV), or Echo-Particle Image Velocimetry (echo-PIV) by analogy with the popular optical technique, has been under development [19]. In this method, regions of two successive B-mode images of a fluid containing scatterers are cross-correlated to calculate 2D velocity vectors [20-22]. Hence the method produces instantaneous, 2D velocity vector fields. UIV has been used to study flow in straight [23, 24] and curved tubes [25], vessel phantoms [26] and vortex phantoms [27], and for preliminary *in vivo* investigations in healthy arteries [26, 28-30] and the heart [28, 31].

In optical PIV, images are acquired as snapshot pairs through the use of specialised cameras and the inter-frame time, Δt , can be freely chosen. In contrast, the B-mode ultrasound images used in UIV are formed by reading out data from groups of transducer elements, to produce image lines

sequentially, and Δt is the same as the acquisition frame rate of the system. So, in UIV, Δt is determined by the speed of sound, depth, and the number of lines in the image [25].

Zhou *et al* [32] showed how the motion of the scatterers and the sweeping of the ultrasound beam across the frame interact to produce potentially large errors in the velocity measurements. Zhou *et al* [32] also showed how to correct for beam sweeping so that errors of just a few per cent are achieved.

Despite this advance, a problem still occurs when the velocity of the scatterers approaches that of the beam sweep. This upper limit on the measureable velocity is well known [26, 33], with authors reporting velocity measurements up to around 70 cm/s [30]. Restricting the field of view [34], or skipping lines [35], can increase the frame rate but at the expense of reduced image width or detail. Leow *et al* [36] used plane wave imaging, in which the whole image frame is captured simultaneously, to achieve frame rates of 1000 Hz and measured velocities up to 80 cm/s. Other examples of the use of high frame rates achieved with plane wave imaging are: UIV of the common carotid [37]; vector Doppler of healthy and diseased carotid arteries [38]; and transverse oscillation of carotid and brachial arteries [39]. However, the hardware used for plane wave imaging is specialist and not widely available.

Poelma and Fraser [40] showed how interleaving images can increase the dynamic range up to at least 140 cm/s while maintaining the full image. The technique works by halving the beam sweep speed so that there is time in between each line of the first image to produce a line of the second image. The moment when the second frame is started can be freely chosen, thus re-introducing control of the critical inter-frame time Δt . The acquisition rate for each image pair is the same as for two images in a conventional sequence; however, the time needed for one image is doubled. Correcting the velocities as described by Zhou *et al* [32] is therefore crucial, but provided this is done measurement of substantially higher velocities is achievable.

To date the interleaved method has not been tested on pulsatile flows, which have periods of accelerating and decelerating flow. As discussed by Zhou *et al* [32] their velocity correction for the ultrasound beam sweep does not account for accelerating ultrasound scatterers. The aims of this work were then to devise and implement a new UIV velocity correction method for accelerating flows, and to compare velocity measurements and derived volumetric flow rates, made using our interleaved UIV method, with Doppler ultrasound velocity and transit-time flow rate measurements. The flows were chosen to be representative of those found in arteries during health and disease. The influence of Δt was investigated, and shown to be particularly important during the deceleration

phase. The optimum Δt was used when subsequently employing the method to measure aortic blood velocity in the rabbit.

2. Methods

2.1 *In vitro* experiments

The experimental facility was similar to that used in earlier studies (see [32]), but adapted to produce pulsatile flow. A 5 mm internal diameter latex tube (098 XA/XB, Primeline Industries, Denver, CO, US) was held straight in a tank of water with an acoustic absorber below. The working fluid was either water or 35% w/w glycerol in water. The fluid was seeded with SonoVue® contrast medium (Bracco UK Ltd), which consists of 1–7 μm encapsulated microbubbles of SF_6 gas.

Pulsatile flow was produced by a positive displacement pump (Harvard Instruments Pulsatile Blood Pump for Rabbits, model 1405). The pump was set to a frequency of either 60 or 200 beats per minute (bpm) (to mimic either human or rabbit heart rates) and the stroke volume was adjusted to give a maximum velocity of either 1.1, 1.6 or 1.8 m/s. The flow waveforms were intended to be typical of the types of flows found in arteries, rather than matching any specific artery. The instantaneous flow rate was measured using a transit-time ultrasound flow probe (Transonic TS420) connected to a laptop running NOTOCORD-hem software (Notocord, France) which was used to perform an ensemble average of 10 cycles, aligned by the ‘foot’ of the waveform. The mean flow rate was also obtained using a stopwatch and measuring cylinder.

An Ultrasonix RP500 (Ultrasonix Medical Corporation, Canada), with a linear 128 element, 38 mm long 5–14 MHz transducer (LP14-5/38) was used to obtain radio frequency data. The Sonix software was used for positioning the transducer, which was placed at the downstream end of the tube, allowing an entrance length of 50 cm (100 diameters). The entrance length for pulsatile flow can be estimated as $L/D = 0.049 \text{ Re}_{\text{ta}}$, where Re_{ta} is the Reynolds number based on the time averaged cross sectional mean velocity [41]. This entrance length therefore resulted in fully developed flow for $\text{Re}_{\text{ta}} < 1020$, which was true for all water-glycerol experiments. For UIV data acquisition the transducer was clamped perpendicular to the tube so as to produce an image of a longitudinal section through the mid-plane.

Following the UIV data acquisition, the position of the probe was adjusted such that an image of the same section of the tube was obtained, but with the ultrasound beam at an angle of between 54 and 60° to the tube wall. The Sonix software was again used for positioning the transducer, and to set all of the Doppler ultrasound parameters: a 1 mm Doppler sample volume was positioned in the middle of the tube; the frequency was 6.6 MHz; the wall filter was between 133 and 250 Hz; and the beam was focussed on the sample volume so had a depth of between 1.7 and 2.5 cm. The pulse repetition frequency (between 6.7 and 12.5 kHz) was adjusted to give the maximum velocity resolution without aliasing. Radio frequency data were recorded and post-processed using Matlab to give the Doppler spectrum and an ensemble average of 10 cycles was produced. Experiment parameters are given in Table 1.

2.2 *In vivo* experiment

All procedures complied with the Animals (Scientific Procedures) Act 1986 and were approved by the Local Ethical Review Process Committee of Imperial College London.

A male New Zealand White rabbit (Harlan Laboratories, UK) was kept in a pen in a rabbit-only room to acclimatise for 2 weeks under normal diet (including plain alfalfa pellets, hay and carrots). At 2.5 months old (2.33 kg) the rabbit was brought into the procedure room and wrapped in a towel for comfort. Hypnorm (0.3 ml/kg) was injected in the calf muscle. The left ear was shaved and marginal ear vein was cannulated using a 23G butterfly needle connected to a three-way tap with Sonovue (25 mg in 5 ml sodium chloride 0.9 %) and sterile saline solution. Throughout anaesthesia (1 hour) the body temperature was maintained at 37°C by a warming plate. A pulse oximeter was connected to the tail for blood oxygenation and heart rate monitoring. The fur around the abdominal region was shaved and the rabbit was placed on its back for access to the aorta. Ultrasound gel was applied to produce an acoustic window on the abdomen. 20 minutes following Hypnorm injection a syringe pump was used to provide a continuous infusion of Sonovue at a rate of 2 ml/min for 2 mins. Images of the rabbit's abdominal aorta in longitudinal section were acquired using the Ultrasonix system as above. Based on the *in vitro* results, the UIV data were acquired with $\Delta t = 31\tau$ (τ is the time taken to produce a single line of the image, see Section 2.3). A Doppler spectrum was then recorded at the same location on the artery. Upon completion of data acquisition, the rabbit was coming out of anaesthesia. It was given 10 ml sterile saline solution sc and kept in a box until able to walk. Body weight, food and water intake, and general condition were monitored the next day and found normal.

2.3 Interleaved UIV data acquisition and processing

The interleaved imaging technique is outlined here; see Poelma and Fraser [40] for a more detailed explanation. In conventional B-mode imaging the transducer elements are read out simultaneously, but so as to produce the image lines sequentially. So the image is produced starting from one side of the transducer and progressing line by line until the full image has been created. The time taken to produce each line, τ , is governed by the transducer response time (time interval between finishing receiving signals from one pulse and starting the next), t_r , the image depth, d , and the speed of sound, c :

$$\tau = t_r + 2d/c \quad (1)$$

The transducer response time was $23.5 \mu\text{s}$ [32]. The time taken to produce the whole image is $J\tau$, where J is the total number of elements, and therefore lines in the image, which is 128 for our transducer. After sweeping across the elements to form this image, the second image is formed in the same way. The time difference between scanning a single line in the first image and the same line in the second image is the inter-frame time, $\Delta t = J\tau$. In interleaved imaging (Fig 1), the first image is recorded using only the odd time steps ($i = 1, 3, \dots$) so line j is recorded at time step $i = 2j - 1$. The even timesteps ($i = 2, 4, \dots$) are used to record the second image frame of the pair. The start time for recording the second image can be any one of the even timesteps; in Fig 1, for example, it is timestep 6. The time difference between scanning the same line in the two successive frames is then $\Delta t = m\tau$, where m is the number of timesteps between the start of the acquisition of the two frames - $m = 5$ in the example.

Using interleaved imaging, the time taken to record a single image is double that of conventional imaging, $T = 2J\tau$. In our current implementation it is longer than double, since some of the time steps at the beginning and end of recording the pair are empty (Fig 1). However, the sequence could be arranged so that the next image pair begins in the empty timesteps at the end of the current pair, to eliminate any wasted time. The inter-frame time is significantly reduced, compared to conventional imaging, and is variable $\Delta t = m\tau$. The theoretical lower limit of Δt is τ , which would require an image line to be recorded twice before moving to the next line.

To implement interleaved imaging, the Ultrasonix RP500 was programmed using a Matlab interface to the Texo software development toolkit. This Matlab interface was developed by Dr Jean Martial Mari (University of French Polynesia) and details are at www.ultrasonix.com/wikisonix.

Images of pulsatile flow were acquired with depths of 2 cm and 6 cm. Values of Δt were between 7τ and 63τ , corresponding to 0.35 – 3.1 ms (2 cm) or 0.71 – 6.4 ms (6 cm). A conventional imaging sequence was also used for comparison, and for that Δt was 128τ , corresponding to 6.3 ms (2 cm) or 13.0 ms (6 cm). The direction of the beam sweep was chosen to be opposite to that of the mean flow (see below). Data were acquired for 25 s, giving between 64 and 165 image pairs depending on depth and Δt . The 25 s data acquisition time gave 25 pulse cycles for the 60 bpm flow and 80 pulse cycles for the 200 bpm flow.

Data were processed using a multi-pass PIV algorithm that uses image deformation and correlation averaging [42]. The analysis started with interrogation areas (IAs) of 32×64 pixels and decreased to 4×8 pixels with 50 % overlap for the final iterations. This corresponded to a spatial resolution of $0.59 \times 0.08 \text{ mm}^2$ in the x and y directions respectively. Since the direction of the flow was in the x direction the IAs were larger in this direction, and smaller in the y direction in which there were velocity gradients. As the flow was time-dependant, the correlation was ensemble averaged at each phase with a temporal resolution of 50 /pulse cycle, which gave 50 Hz or 166 Hz for the 60 and 200 bpm pulse rates respectively. To correctly bin the data for the correlation averaging the PIV algorithm consisted of two stages. In the first stage a single PIV iteration was used to produce a rough estimate for the velocity magnitude in the region of interest. This signal was then used to align each of the cycles by using a cross correlation to determine the phase shift. This phase shift was then used to align the raw data before the second, multi-iteration PIV algorithm was applied.

The new velocity correction method was derived as follows. Following the analysis by Zhou *et al* [32], the position in the x-direction of a scatterer in an unsteady flow at the time it is detected by the sweeping ultrasound beam can be equated with the corresponding position of the beam (Fig 2):

$$x + V_x t + \frac{1}{2} a_x t^2 = x' \quad (2)$$

In this equation x , V_x and a_x are the x-components of the position, velocity and acceleration of the scatterer at the beginning of the image frame acquisition, and x' is the position in the image which is also the position of the beam. If the ultrasound beam sweep velocity is V_s , the time at which the scatterer and beam meet is $t = x'/V_s$:

$$x + \frac{V_x x'}{V_s} + \frac{a_x}{2} \left(\frac{x'}{V_s} \right)^2 = x' \quad (3)$$

Differentiation with respect to t then gives:

$$V_x \left(1 + \frac{V_x'}{V_s} \right) + a_x \left(\frac{x'}{V_s} + \frac{x'V_x'}{V_s^2} \right) + \frac{1}{2} \frac{da_x}{dt} \left(\frac{x'}{V_s} \right)^2 = V_x' \quad (4)$$

The velocity measurements are made over a finite time period, so at the time n the real velocity in the x -direction can be written $V_{x n}$, the real acceleration $a_{x n}$ and the measured velocity $V_{x n}'$:

$$V_{x n} \left(1 + \frac{V_{x n}'}{V_s} \right) + a_{x n} \left(\frac{x'}{V_s} + \frac{x'V_{x n}'}{V_s^2} \right) + \frac{1}{2} \frac{da_{x n}}{dt} \left(\frac{x'}{V_s} \right)^2 = V_{x n}' \quad (5)$$

Then, since the velocity measurements are made over time we can introduce approximations for $a_{x n}$ and $\frac{da_{x n}}{dt}$ as functions of V_x . For example, 2nd order central difference approximations are:

$$a_{x n} = \frac{V_{x n+1} - V_{x n-1}}{2\Delta T} \quad (6)$$

$$\frac{da_{x n}}{dt} = \frac{V_{x n+1} - 2V_{x n} - V_{x n-1}}{2\Delta T} \quad (7)$$

where $V_{x n-1}$ and $V_{x n+1}$ are the real velocities at timesteps $n-1$ and $n+1$ respectively, and ΔT is the time between successive image *pairs* (this is not the same as Δt , the time between image frames within the same pair). Making this substitution leads to a set of N simultaneous equations of the form:

$$\begin{aligned} \frac{-x'}{2\Delta T V_s} \left(-\frac{V_{x n}'}{V_s} - 1 + \frac{x'}{V_s \Delta T} \right) V_{x n-1} + \left(\frac{V_{x n}'}{V_s} + 1 - \left(\frac{x'}{V_s \Delta T} \right)^2 \right) V_{x n} \\ + \frac{x'}{2\Delta T V_s} \left(\frac{V_{x n}'}{V_s} + 1 + \frac{x'}{V_s \Delta T} \right) V_{x n+1} = V_{x n}' \end{aligned} \quad (8)$$

which relate the real velocity V_x to the velocity V_x' found from processing the images using the PIV algorithm. We used 4th order approximations for $a_{x n}$ and $\frac{da_{x n}}{dt}$ to achieve smoother results, but the method is the same as for the 2nd order approximations shown above. Since we are assuming that the flow is periodic - this assumption underlies the use of ensemble averaging - there are a total of N unknowns so the system of equations can be solved. This was done using matrix left division (“\”) in Matlab. The y -component of velocity V_y was found in the same way, and so the calculation of V_y depends on V_x' .

In steady flow, eqn (4) simplifies and the equation for the x -velocity correction resulting from the beam sweep is:

$$V_x \approx \frac{V_s V_{x'}}{V_s + V_{x'}} \equiv V_{x \text{ steady}} \quad (9)$$

To assess the error associated with the acceleration part of the beam sweep correction, the velocity found using eqn (9) was compared with that from eqn (8).

Rearranging the velocity correction equation for V_x , eqn (9), gives an equation for the observed velocity $V_{x'}$, dependent on the real velocity of the scatterer and the beam sweep:

$$V_{x'} \approx \frac{V_s V_x}{V_s - V_x} \quad (10)$$

It is clear that as scatterer velocities approach the beam sweep velocity, $V_{x'}$ will become infinite. That is, the displacement of the scatterers between the images is larger than the size of the interrogation area, even for the largest possible areas, because the sweeping beam cannot 'catch up' with the moving scatterers. This is true even for interleaved imaging. However, if the direction of the beam sweep is opposite to that of the scatterers, there is theoretically no maximum V_x ; the denominator in (10) simply gets bigger. This is why the direction of the beam sweep was chosen to be opposite to that of the mean flow.

3. Results

All data and code are available for download [43].

3.1 *In vitro* experiments

3.1.1 Baseline flow condition

Fig 3 shows results for the straight tube containing glycerol-water, with a pulsatile frequency of 60 bpm and peak velocity around 1 m/s (flow "a", parameters typical of healthy arterial flow in the human). The image depth was 2 cm and $\Delta t = 31\tau$. The velocity vector plots show that the velocity can be measured across the whole width of the transducer. The resolution of the vector plots is higher than illustrated, for clarity only every 8th vector in the x direction and every 4th vector in the y direction is shown.

Fig 3E shows a comparison of the velocity in the centre of the tube with the Doppler spectrum obtained at the same position. The Doppler spectrum shown is the ensemble average of 50 individual pulse cycles. The sample volume for the UIV (also shown in Fig 3E) encompassed the width of the image but had the same height as the Doppler sample volume. Although the temporal resolution of the Doppler measurements is much higher than the UIV ones, there is still excellent agreement between the magnitudes of the two velocity waves. To assess the difference, the maximum of the Doppler spectrum was calculated throughout the cycle and the mean of the absolute difference between this and the average UIV was found. The difference was 7.9 cm/s or 7 % of the peak velocity (Table 2). The maximum of the Doppler spectrum was used because it is less sensitive to

location than the mean, and provided the Doppler angle is less than 60° , the error is less than 30 % [44].

The flow waveform was estimated from the UIV (Fig 3F). The inner walls of the tube were detected automatically and used to predict the centre of the tube. The radial position of each velocity vector was then found and the velocity was integrated cylindrically to find the flow rate. A comparison between this flow rate and the flow rate measured using the transit time flow meter (Fig 3F) shows a very good agreement through the majority of the pulse cycle (mean absolute difference is 60 ml/min or 4.4 % of peak flow, Table 2). However, due to the temporal resolution of the UIV, the first peak is not captured. This error could potentially be resolved by acquiring data for longer, and then using more phase bins for the correlation average in the PIV algorithm, however, for *in vivo* measurements temporal flow variations limit the desirable acquisition time. There is also a difference in the shape of the main peak.

3.1.2 Influence of inter-frame time Δt

Reducing Δt to $\Delta t = 15\tau$ had the effect of smoothing out the velocity waveform, with the result that small peaks, such as the one occurring at 0.16 s, were not detected, and the main peak was reduced (Fig 4A). The mean absolute difference compared to Doppler was 11 cm/s and compared to transit time flow rate was 84 ml/min. This happens because when Δt is smaller the displacement of the scatters between the two frames is smaller and hence the velocity resolution is reduced. Conversely increasing Δt increases the velocity resolution; with $\Delta t = 95\tau$ (Fig 4B) the UIV velocity waveform neatly outlined the top of the Doppler spectrum during the acceleration and peak phases of the pulse cycle. However, during the deceleration phase it is clear that the longer Δt reduces the quality of the velocity measurement (mean absolute difference compared to Doppler was 11 cm/s and compared to transit time flow rate was 82 ml/min). During deceleration the flow is transitional and the scatterers gain random 3D velocity fluctuations. The in-plane velocity fluctuations mean the relative positions of the scatterers differ between the image frames, and the out-of-plane velocity component means that the scatterers might not appear in the second frame. These two effects both result in an increasing loss of correlation for longer Δt [45]. This random process results in a spread of measured velocities. The effect is even more noticeable in the results using the conventional, rather than interleaved, imaging method, which has $\Delta t = 128\tau$ (Fig 4C). The flow rate measurements with lower Δt agree with the transit time flow meter however for $\Delta t > 79\tau$ the flow is underestimated during deceleration at around 0.5 s (Fig 4D). The difference between velocity profiles during peak velocity and during deceleration is shown in Fig 4E and F.

3.1.3 Influence of increased pulse cycle frequency

The frequency of the pulse cycle was then increased to 200 bpm (flow “b”, Fig 5). The centreline velocities for all the values of Δt are very similar, although the longer values do give slightly better velocity resolution (most noticeably in the second peak.) The peak velocity was underestimated at 0.86 m/s, compared to the 1.1 m/s obtained by Doppler. The short duration of this velocity ‘spike’ in the Doppler spectrum is likely to explain why it was not seen in either the UIV or flow probe results. There was no signal loss in the UIV results during deceleration, which could be because the deceleration phase is so short that there is not enough time for transition to occur [46].

3.1.4 Influence of increased peak velocity

While maintaining a pulse rate of 200 bpm, the peak velocity was then increased to 1.8 m/s (flow “c”, Fig 6). For this flow waveform, the main peak in the velocity wave was well matched by the interleaved UIV for values of Δt between 7τ and 63τ , whereas conventional UIV could not measure these fast velocities. None of the UIV methods could reproduce the negative velocities in the Doppler signal at around 0.12 s.

3.1.5 Influence of increased Reynold’s number

To further investigate the abilities of UIV during flow deceleration the working fluid was switched to water (flows “d” and “e”, Fig 7). The flow waveforms in Figs 7E and 4D are very similar (maximum velocity 1.1 m/s, minimum velocity -0.2 m/s) however Re_{ta} was 2120 in the case of water, compared to 500 for water-glycerol. Comparing these flow waveforms it is clear that with the lower viscosity fluid the signal ‘dropout’ occurs at a shorter Δt (63τ , compared with 79τ for the higher viscosity fluid). In the low viscosity (high Reynolds number) case the transition to turbulence occurs sooner, and/or leads to more turbulent fluctuations and hence more signal loss. Increasing the peak velocity to 1.6 m/s required $\Delta t \leq 15\tau$ to properly capture the velocity and flow waveforms throughout the entire pulse cycle.

3.1.6 Influence of increased image depth

Increasing the image depth (flow “e”, Fig 8) did not cause any problems for the velocity measurement provided that the value for Δt remained similar. For example, the flow waveforms with the depth of 2 cm and $\Delta t = 15\tau$ (0.74 ms) and 63τ (3.12 ms) were very similar to those with depth of 6 cm and $\Delta t = 7\tau$ (0.71 ms) and 31τ (3.14 ms) respectively. Likewise the waveform with depth 6 cm and $\Delta t = 63\tau$ (6.39 ms) was similar to that obtained from the conventional UIV method which has $\Delta t = 128\tau$ (6.33 ms).

3.1.7 Comparison with theoretical velocity profiles

Velocity profiles were produced by averaging the velocity along the length of the tube. The change in the velocity profile throughout the cycle is shown in Fig 3G. Womersley solved the velocity field for pulsatile flow in a long straight tube when the pressure gradient is known [47] and using that theory the profile can be calculated from either the centerline or mean velocity [48]. The tube used here was flexible so an exact match is not expected however, for interest, we calculated the Womersley profiles using the flow rate from the transit time flow probe and the mean tube radius measured from the B-mode images.

The resulting theoretical profiles for flow “a” are shown in Fig 3G and the mean absolute difference between Womersley and UIV was found to be 6.4 cm/s or 6 % of the peak velocity. This Womersley comparison was repeated for the remaining flows and the mean absolute difference between the Womersley profiles and the UIV profiles was 9-11 % of the peak velocity.

3.1.8 Assessment of variability in the measurements and the flow

The UIV algorithm uses correlation averaging (as explained in section 2.3 and [42]) over a number of pulse cycles which means there is no intrinsic estimate of the variability in the results. An estimate of the upper limit on the variability in the results was produced in the following way. The UIV processing was repeated using just 30 % of the original data, this corresponded to about 7.5 pulse cycles at 60 bpm and 21 pulse cycles at 200 bpm. The first 30 % was used to produce one time dependent velocity field, the next 30 % produced a second velocity field, and the third 30% produced a third velocity field. The variability between these independent velocity fields was assessed by comparing the velocity in the sample volume and the flow rate, as presented above for the full data. The mean standard deviation in the velocity was between 1 and 4 cm/s (1-6 % of peak velocity) and the mean standard deviation in the flow rate was 2-5 % of the peak flow rate. The actual variability in the flow was assessed using the standard deviation of the flow rate measured using the transit time flow meter and this was 0.8-1.5 % of the peak flow rate.

3.2 *In vivo* experiment

The UIV data were acquired for 25 s but due to motion, possibly breathing or difficulty holding the transducer in position, only 10 s of the data were usable. This corresponded to around 40 cardiac cycles.

Velocity vectors are plotted for the entire field of view at 0.036 s in Fig 9A, and for a sub-region of interest for further time steps in Figs 9(C-E). Note that for clarity not all of the vectors have been

plotted; the resolution of the full vector field is twice that for the straight tube experiments in the x direction ($0.30 \times 0.08 \text{ mm}^2$, x × y directions).

The shape of the velocity waveform measured with UIV (Fig 9B) was in good agreement with the Doppler spectrum and the mean absolute difference was 7 cm/s or 9 % of peak velocity. Peak systolic velocities were 77 cm/s and 82 cm/s with UIV and Doppler respectively. The difference is likely to be due to the practical difficulty of aligning the transducer with the centre of the aorta and holding it steady, as well as the known errors in Doppler ultrasound; *in vivo* Doppler velocity measurement errors may be up to 75 % even with an experienced sonographer [10, 49]. Peak systolic flow was calculated to be 175 ml/min, within the range found for similar anaesthetised rabbits [50].

The variability in the flow was assessed using the Doppler spectrum. The maximum velocity at peak systole was recorded for 10 successive cycles and the standard deviation was 3 cm/s or 3.5 % of the peak systolic velocity.

4. Discussion

In this work, a method for using conventional clinical ultrasound hardware to map arterial velocities has been developed. The method is based on interleaved imaging [40], which extends the dynamic range of UIV by enabling shorter Δt without reducing image resolution or field of view. Here the interleaved method has been extended to include phase-based correlation averaging [42] and a method of correcting the velocities for errors introduced by accelerating flows and the sweeping ultrasound beam, a problem first identified by Zhou *et al* [32].

Interleaved UIV was used to measure velocity and flow in a straight tube phantom with a variety of flow waveforms. These experiments demonstrated that peak velocities of 1.8 m/s could be measured for heart rates of 200 bpm. With a heart rate of 60 bpm the velocities could be accurately measured throughout the cardiac cycle, even for the highest peak velocities. Both of the in-plane velocity components were measured across the entire 38 mm width of the transducer. While peak velocities in healthy arteries are usually of the order 1 m/s, in diseased arteries they can be much higher: for example with a carotid stenosis of 50-70 % the velocity is in the range 125-230 cm/s [2]. Peak velocities measured using conventional UIV are around 70 cm/s [30], although 110 cm/s was obtained in the present study.

This work has revealed a previously unreported problem with conventional UIV: the measurement of velocity in decelerating flow. The transitional nature of decelerating flows means the scatterers

become decorrelated within shorter times and hence require a shorter Δt . Conventional UIV was unable to measure velocity for the complete deceleration phase of the 1.1 m/s peak velocity, 60 bpm flow. However, reducing Δt to $\leq 63 \tau$ enabled accurate measurement of the velocity throughout the decelerating phase. Increasing the Reynolds number required even smaller Δt .

4.1 Acceleration correction

The problem of velocity errors introduced by ultrasound beam sweeping has been reported before [25] and velocity correction methods for the velocity components parallel and perpendicular to the direction of the ultrasound beam sweep have been derived for steady flow [32]. Zhou *et al* [32] also reported that there is an additional error in accelerating flows but did not give a correction method. Here we have introduced a method for correcting this additional error which is based on finding the acceleration from the difference between consecutive velocity measurements.

To investigate the difference made to the velocity measurements by the new correction method, the results were compared with those found using the steady flow velocity correction method in eqn 9 [32] (Fig 10). During the flow acceleration phase the steady flow method overestimates the velocity compared with the new method, whereas during deceleration the opposite is true. The new correction method can be thought of as containing two parts: the steady velocity correction, $V_{x \text{ steady}} - V_x'$, which was found using eqn 10, and an additional correction for the acceleration $V_x - V_{x \text{ steady}}$. Since in this experiment the direction of the flow is always opposite to the ultrasound beam sweep direction, the steady velocity correction is positive throughout the cycle. The direction of the additional acceleration correction is negative during acceleration and positive during deceleration. At the phases where there is a low velocity, but significant acceleration, the magnitude of the additional acceleration correction can be greater than the steady velocity correction. The additional acceleration correction varies with position in the image, increasing in magnitude in the direction of the beam sweep. The magnitude of the total correction, $V_x - V_x'$, was generally around 10 % of the real velocity, V_x , but could reach over 60 %.

4.2 Limitations

The flow waveforms used in the phantom experiments were intended to be representative of arterial flows in the human and rabbit, rather than exactly matching specific arteries. The flows had large in-plane velocity components. UIV suffers limitations in the presence of large out-of-plane velocity components since the scatterers leave the field of view [45]. With the reduction in Δt , the

interleaved method should significantly reduce these limitations, similar to the improvement shown in measuring decelerating flow. For more complicated arterial geometries such as curves, bifurcations and diseased regions, the flow fields are more complicated and there is a greater need for two velocity components to describe the flow [51, 52]. These flows also have significant out of plane components. The benefits of UIV, and particularly interlaced UIV which has the ability to shorten Δt , will be substantial in these types of flows and investigating them is an important next step. The introduction of 3D ultrasound could provide three components of the flow.

The method implemented here relied on post processing to ensemble average the data. The ensemble averaging has some limitations. The flow phantom data was acquired for 25 s which is significantly longer than a single Doppler measurement. To assess the impact of using less data for the ensemble average the processing was repeated successively using 10% less data each time. With only 30% of the original data (about 7.5 s) the differences between the UIV results and the validation techniques were slightly higher than with the full data: the difference in the velocity compared with the Doppler spectrum was 9-10 % of peak velocity and the difference in the flow rate compared with the transit time flow meter was 5-7 % of peak flow. Another potential problem relates to arrhythmias and more unusual measurement situations such as cardiac arrest. Development of a graphical user interface with real time information would be very helpful for *in vivo* studies. The real time system should allow the correlation to be built up over time, similar to 'persistence', or image averaging, that is currently implemented on ultrasound scanners. This could be done using a phase locked sliding correlation average.

Both spectral Doppler ultrasound and transit time flow measurement have associated errors, such as spectral broadening and manual angle correction. The combined use of both techniques for validation provides greater certainty in the results.

5. Conclusions

Interleaved UIV provides time resolved, angle independent and full field-of-view maps of *in vivo* velocities using conventional ultrasound hardware. Provided the appropriate correction is implemented to account for ultrasound beam sweeping, including the new acceleration correction method introduced here, the measurements are accurate: percentage errors were 5-10 % compared with spectral Doppler and 2-9 % compared with the transit time flow meter. Conventional UIV measurements have been found to be unreliable during flow deceleration, even for moderate

decelerations. Interlaced UIV successfully reduces the inter-frame time, facilitating measurements of pulsatile blood velocities up to at least 1.8 m/s, including during the deceleration phase. This could enable its use in clinical assessments and thus increase diagnostic accuracy, and allow its use in physiological research, opening up new possibilities for studying the links between blood flow and disease progression.

Author Contributions

The experiments were conducted in the Department of Bioengineering at Imperial College London and data analysis in the Department of Mechanical Engineering at University of Bath. KHF designed the study, conducted all experiments and interpreted the data; CP provided UIV code, technical knowledge and data interpretation; BZ assisted with *in vitro* experiments and velocity corrections; EB conducted *in vivo* experiments with KHF; MXT and PDW helped with the experiment design and analysis of results. All authors contributed to drafting and revising the manuscript and gave final approval for publication.

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Competing interests

None of the authors have competing interests.

Tables

#	fluid	Frequency (bpm)	V_{\max} (m/s)	V_{\min} (m/s)	Q_{\max} (ml/min)	Q_{ta} (ml/min)	Re_{ta}
a	water-glycerol	60	1.1	-0.2	1331	385	500
b	water-glycerol	200	1.1	-0.2	1240	365	480
c	water-glycerol	200	1.8	-0.3	2460	750	990
d	water	60	1.1	-0.2	1445	500	2120
e	water	60	1.6	-0.4	2279	635	2695

Table 1: Flow related parameters for the *in vitro* experiments. The viscosity, μ , and density, ρ , of 35 % w/w glycerol in water were assumed to be 0.003 Pa s and 1088 kg/m³ [Glycerine Producers 53]. The frequency given is the frequency set by the pump. V_{\max} and V_{\min} are the maximum and minimum velocities measured from the Doppler spectrum. Q_{\max} and Q_{ta} are the maximum and cycle averaged flow rates measured by the transit time flow probe. Re_{ta} is the cycle averaged Reynolds number $Re_{ta} = \frac{Q_{ta} D \rho}{A \mu}$ where D and A are the diameter and cross sectional area of the pipe.

Flow #	ΔT	maximum Doppler velocity		transit time flow rate	
	τ	m/s	%	ml/min	%
a	31	0.079	7.5	60	4.4
b	31	0.078	9.0	80	6.2
c	7	0.18	10	166	8.7
d	15	data corrupted		33	2.2
e	7	0.086	5.4	127	5.9

Table 2: Differences between UIV measurements and validation measurements. Table shows the data for the optimum ΔT . For each method the mean absolute difference between the UIV measurement and the validation measurement is given, as well as the value as a percentage of the maximum over the pulse cycle. For the Doppler comparison the average UIV value in the sample volume was compared with the maximum in the Doppler spectrum.

References

- [1] von Reutern, G.-M., Goertler, M.-W., Bornstein, N.M., Del Sette, M., Evans, D.H., Hetzel, A., Kaps, M., Perren, F., Razumovsky, A., von Reutern, M., et al. 2012 Grading Carotid Stenosis Using Ultrasonic Methods. *Stroke* **43**, 916-921. (doi:10.1161/strokeaha.111.636084).
- [2] Hathout, G.M., Fink, J.R., El-Saden, S.M. & Grant, E.G. 2005 Sonographic NASCET index: A new Doppler parameter for assessment of internal carotid artery stenosis. *American Journal of Neuroradiology* **26**, 68-75.
- [3] Secchi, F., Iozzelli, A., Papini, G.D.E., Aliprandi, A., Di Leo, G. & Sardanelli, F. 2009 MR imaging of aortic coarctation. *Radiol. Med.* **114**, 524-537. (doi:10.1007/s11547-009-0386-6).
- [4] Bermejo, J., Martinez-Legazpi, P. & del Alamo, J.C. 2015 The Clinical Assessment of Intraventricular Flows. In *Annual Review of Fluid Mechanics, Vol 47* (eds. S.H. Davis & P. Moin), pp. 315-342. Palo Alto, Annual Reviews.
- [5] Sotiropoulos, F., Le, T.B. & Gilmanov, A. 2016 Fluid Mechanics of Heart Valves and Their Replacements. In *Annual Review of Fluid Mechanics, Vol 48* (eds. S.H. Davis & P. Moin), pp. 259-283.
- [6] Zhong, Y., Zhu, W., Li, C.M. & Rao, L. 2016 Assessment of cardiac dysfunction by dissipative energy loss derived from vector flow mapping. *Journal of Cardiology* **67**, 122-122. (doi:10.1016/j.jcc.2015.06.004).
- [7] Lin, J.B., Phillips, E.H., Riggins, T.E., Sangha, G.S., Chakraborty, S., Lee, J.Y., Lycke, R.J., Hernandez, C.L., Soepriatna, A.H., Thorne, B.R.H., et al. 2015 Imaging of Small Animal Peripheral Artery Disease Models: Recent Advancements and Translational Potential. *Int. J. Mol. Sci.* **16**, 11131-11177. (doi:10.3390/ijms160511131).
- [8] Kidher, E., Cheng, Z., Jarral, O.A., O'Regan, D.P., Xu, X.Y. & Athanasiou, T. 2014 In-vivo assessment of the morphology and hemodynamic functions of the BioValsalva (TM) composite valve-conduit graft using cardiac magnetic resonance imaging and computational modelling technology. *J. Cardiothorac. Surg.* **9**, 11. (doi:10.1186/s13019-014-0193-6).
- [9] Evans, D.H. & McDicken, W.N. 2000 *Doppler ultrasound: Physics, Instrumentation and Signal Processing*. Chichester, John Wiley.
- [10] Lui, E.Y.L., Steinman, A.H., Cobbold, R.S.C. & Johnston, K.W. 2005 Human factors as a source of error in peak Doppler velocity measurement. *Journal of Vascular Surgery* **42**, 972.e971-972.e910. (doi:<http://dx.doi.org/10.1016/j.jvs.2005.07.014>).
- [11] Logason, K., Barlin, T., Jonsson, M.L., Bostrom, A., Hardemark, H.G. & Karacagil, S. 2001 The importance of Doppler angle of insonation on differentiation between 50-69% and 70-99% carotid artery stenosis. *Eur. J. Vasc. Endovasc. Surg.* **21**, 311-313. (doi:10.1053/ejvs.2001.1331).
- [12] Department", S.P.C.S. 2010 UK Audit of Vascular Surgical Services & Carotid Endarterectomy. (Royal College of Physicians of London).
- [13] Monteiro, A., Santos, R., Ferreira, C., Costa, A. & Azevedo, E. 2014 Internal carotid artery stenosis: validation of Doppler velocimetric criteria.
- [14] Taylor, C.A. & Draney, M.T. 2004 Experimental and computational methods in cardiovascular fluid mechanics. *Annual Review of Fluid Mechanics* **36**, 197-231. (doi:10.1146/annurev.fluid.36.050802.121944).
- [15] Evans, D.H. 2010 Colour flow and motion imaging. *Proceedings of the Institution of Mechanical Engineers Part H-Journal of Engineering in Medicine* **224**, 241-253. (doi:10.1243/09544119jeim599).
- [16] Evans, D.H., Jensen, J.A. & Nielsen, M.B. 2011 Ultrasonic colour Doppler imaging. *Interface Focus* **1**, 490-502. (doi:10.1098/rsfs.2011.0017).
- [17] Bohs, L.N., Geiman, B.J., Anderson, M.E., Gebhart, S.C. & Trahey, G.E. 2000 Speckle tracking for multi-dimensional flow estimation. *Ultrasonics* **38**, 369-375. (doi:10.1016/s0041-624x(99)00182-1).
- [18] Hoskins, P.R. & Kenwright, D.A. 2015 Recent developments in vascular ultrasound technology. *Ultrasound* **23**, 158-165. (doi:10.1177/1742271x15578778).

- [19] Crapper, M., Bruce, T. & Gouble, C. 2000 Flow field visualization of sediment-laden flow using ultrasonic imaging. *Dynamics of Atmospheres and Oceans* **31**, 233-245. (doi:10.1016/s0377-0265(99)00035-4).
- [20] Jensen, J.A., Nikolov, S.I., Yu, A.C.H. & Garcia, D. 2016 Ultrasound Vector Flow Imaging—Part II: Parallel Systems. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control* **63**, 1722-1732. (doi:10.1109/TUFFC.2016.2598180).
- [21] Jensen, J.A., Nikolov, S.I., Yu, A.C.H. & Garcia, D. 2016 Ultrasound Vector Flow Imaging—Part I: Sequential Systems. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control* **63**, 1704-1721. (doi:10.1109/TUFFC.2016.2600763).
- [22] Poelma, C. 2016 Ultrasound Imaging Velocimetry: a review. *Experiments in Fluids* **58**, 3. (doi:10.1007/s00348-016-2283-9).
- [23] Kim, H.B., Hertzberg, J., Lanning, C. & Shandas, R. 2004 Noninvasive measurement of steady and pulsating velocity profiles and shear rates in arteries using echo PIV: In vitro validation studies. *Annals of Biomedical Engineering* **32**, 1067-1076.
- [24] Kim, H.B., Hertzberg, J.R. & Shandas, R. 2004 Development and validation of echo PIV. *Experiments in Fluids* **36**, 455-462. (doi:10.1007/s00348-003-0743-5).
- [25] Poelma, C., Mari, J.M., Foin, N., Tang, M.X., Krams, R., Caro, C.G., Weinberg, P.D. & Westerweel, J. 2011 3D Flow reconstruction using ultrasound PIV. *Experiments in Fluids* **50**, 777-785. (doi:10.1007/s00348-009-0781-8).
- [26] Liu, L., Zheng, H., Williams, L., Zhang, F., Wang, R., Hertzberg, J. & Shandas, R. 2008 Development of a custom-designed echo particle image velocimetry system for multi-component hemodynamic measurements: system characterization and initial experimental results. *Physics in Medicine and Biology* **53**, 1397-1412. (doi:10.1088/0031-9155/53/5/015).
- [27] Westerdale, J., Belohlavek, M., McMahon, E.M., Jiamsripong, P., Heys, J.J. & Milano, M. 2011 Flow Velocity Vector Fields by Ultrasound Particle Imaging Velocimetry In Vitro Comparison With Optical Flow Velocimetry. *Journal of Ultrasound in Medicine* **30**, 187-195.
- [28] de Azevedo, B.A., Azevedo, L.F.A., Nunes, R. & Nobrega, A.C.L. 2015 In vivo blood velocity measurements with particle image velocimetry in echocardiography using spontaneous contrast. *Journal of the Brazilian Society of Mechanical Sciences and Engineering* **37**, 559-569. (doi:10.1007/s40430-014-0205-4).
- [29] Qian, M., Niu, L., Wang, Y., Jiang, B., Jin, Q., Jiang, C. & Zheng, H. 2010 Measurement of flow velocity fields in small vessel-mimic phantoms and vessels of small animals using micro ultrasonic particle image velocimetry (micro-EPIV). *Physics in Medicine and Biology* **55**, 6069-6088. (doi:10.1088/0031-9155/55/20/003).
- [30] Zhang, F., Lanning, C., Mazzaro, L., Barker, A.J., Gates, P.E., Strain, W.D., Fulford, J., Gosling, O.E., Shore, A.C., Bellenger, N.G., et al. 2011 IN VITRO AND PRELIMINARY IN VIVO VALIDATION OF ECHO PARTICLE IMAGE VELOCIMETRY IN CAROTID VASCULAR IMAGING. *Ultrasound in Medicine and Biology* **37**, 450-464. (doi:10.1016/j.ultrasmedbio.2010.11.017).
- [31] Hong, G.-R., Pedrizzetti, G., Tonti, G., Li, P., Wei, Z., Kim, J.K., Baweja, A., Liu, S., Chung, N., Houle, H., et al. 2008 Characterization and Quantification of Vortex Flow in the Human Left Ventricle by Contrast Echocardiography Using Vector Particle Image Velocimetry. *Jacc-Cardiovascular Imaging* **1**, 705-717. (doi:10.1016/j.jcmg.2008.06.008).
- [32] Zhou, B., Fraser, K.H., Poelma, C., Mari, J.-M., Eckersley, R.J., Weinberg, P.D. & Tang, M.-X. 2013 ULTRASOUND IMAGING VELOCIMETRY: EFFECT OF BEAM SWEEPING ON VELOCITY ESTIMATION. *Ultrasound in Medicine and Biology* **39**, 1672-1681. (doi:10.1016/j.ultrasmedbio.2013.03.003).
- [33] Poelma, C., van der Mijle, R.M.E., Mari, J.M., Tang, M.X., Weinberg, P.D. & Westerweel, J. 2012 Ultrasound imaging velocimetry: Toward reliable wall shear stress measurements. *European Journal of Mechanics B-Fluids* **35**, 70-75. (doi:10.1016/j.euromechflu.2012.03.003).
- [34] Beulen, B., Bijmens, N., Rutten, M., Brands, P. & van de Vosse, F. 2010 Perpendicular ultrasound velocity measurement by 2D cross correlation of RF data. Part A: validation in a straight tube. *Experiments in Fluids* **49**, 1177-1186. (doi:10.1007/s00348-010-0865-5).

- [35] Walker, A.M., Scott, J., Rival, D.E. & Johnston, C.R. 2014 In vitro post-stenotic flow quantification and validation using echo particle image velocimetry (Echo PIV). *Experiments in Fluids* **55**. (doi:10.1007/s00348-014-1821-6).
- [36] Leow, C.H., Bazigou, E., Eckersley, R.J., Yu, A.C.H., Weinberg, P.D. & Tang, M.-X. 2015 FLOW VELOCITY MAPPING USING CONTRAST ENHANCED HIGH-FRAME-RATE PLANE WAVE ULTRASOUND AND IMAGE TRACKING: METHODS AND INITIAL IN VITRO AND IN VIVO EVALUATION. *Ultrasound in Medicine and Biology* **41**, 2913-2925. (doi:10.1016/j.ultrasmedbio.2015.06.012).
- [37] Udesen, J., Gran, F., Hansen, K.L., Jensen, J.A., Thomsen, C. & Nielsen, M.B. 2008 High frame-rate blood vector velocity imaging using plane waves: Simulations and preliminary experiments. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control* **55**, 1729-1743. (doi:10.1109/TUFFC.2008.858).
- [38] Ekroll, I.K., Swillens, A., Segers, P., Dahl, T., Torp, H. & Lovstakken, L. 2013 Simultaneous quantification of flow and tissue velocities based on multi-angle plane wave imaging. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control* **60**, 727-738. (doi:10.1109/TUFFC.2013.2621).
- [39] Lenge, M., Ramalli, A., Tortoli, P., Cachard, C. & Liebgott, H. 2015 Plane-wave transverse oscillation for high-frame-rate 2-D vector flow imaging. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control* **62**, 2126-2137. (doi:10.1109/TUFFC.2015.007320).
- [40] Poelma, C. & Fraser, K.H. 2013 Enhancing the dynamic range of ultrasound imaging velocimetry using interleaved imaging. *Measurement Science and Technology* **24**. (doi:10.1088/0957-0233/24/11/115701).
- [41] Carpinlioglu, M.O. & Gundogdu, M.Y. 2001 A critical review on pulsatile pipe flow studies directing towards future research topics. *Flow Meas. Instrum.* **12**, 163-174.
- [42] Poelma, C., Vennemann, P., Lindken, R. & Westerweel, J. 2008 In vivo blood flow and wall shear stress measurements in the vitelline network. *Experiments in Fluids* **45**, 703-713. (doi:10.1007/s00348-008-0476-6).
- [43] Fraser, K.H., Poelma, C., Zhou, B., Bazigou, E., Tang, M.X. & Weinberg, P.D. 2016 Data from "Validation of Ultrasound Imaging Velocimetry using interleaved images for measuring arterial blood flow" <http://researchdata.bath.ac.uk/> (embargoed until publication). (
- [44] Hoskins, P.R. 1999 A comparison of single- and dual-beam methods for maximum velocity estimation. *Ultrasound in Medicine & Biology* **25**, 583-592. (doi:[http://dx.doi.org/10.1016/S0301-5629\(98\)00189-6](http://dx.doi.org/10.1016/S0301-5629(98)00189-6)).
- [45] Adrian, R.J. & Westerweel, J. 2011 *Particle Image Velocimetry*, Cambridge University Press; 586 p.
- [46] Trip, R., Kuik, D.J., Westerweel, J. & Poelma, C. 2012 An experimental study of transitional pulsatile pipe flow. *Phys. Fluids* **24**, 17. (doi:10.1063/1.3673611).
- [47] Womersley, J.R. 1955 Method for the calculation of velocity, rate of flow and viscous drag in arteries when the pressure gradient is known. *The Journal of Physiology* **127**, 553-563.
- [48] Blake, J.R., Meagher, S., Fraser, K.H., Easson, W.J. & Hoskins, P.R. 2008 A Method to Estimate Wall Shear Rate with a Clinical Ultrasound Scanner. *Ultrasound in Medicine & Biology* **34**, 760-774. (doi:<http://dx.doi.org/10.1016/j.ultrasmedbio.2007.11.003>).
- [49] Tortoli, P., Lenge, M., Righi, D., Ciuti, G., Liebgott, H. & Ricci, S. 2015 Comparison of Carotid Artery Blood Velocity Measurements by Vector and Standard Doppler Approaches. *Ultrasound in Medicine & Biology* **41**, 1354-1362. (doi:<http://dx.doi.org/10.1016/j.ultrasmedbio.2015.01.008>).
- [50] Alastruey, J., Hunt, A.A.E. & Weinberg, P.D. 2014 Novel wave intensity analysis of arterial pulse wave propagation accounting for peripheral reflections. *Int. J. Numer. Meth. Biomed.* **30**, 249-279.
- [51] Tortoli, P., Michelassi, V., Bambi, G., Guidi, F. & Righi, D. 2003 Interaction between secondary velocities, flow pulsation and vessel morphology in the common carotid artery. *Ultrasound in Medicine & Biology* **29**, 407-415. (doi:[http://dx.doi.org/10.1016/S0301-5629\(02\)00705-6](http://dx.doi.org/10.1016/S0301-5629(02)00705-6)).

- [52] Steinman, D.A. 2004 Image-based Computational Fluid Dynamics: A New Paradigm for Monitoring Hemodynamics and Atherosclerosis. *Current Drug Target -Cardiovascular & Hematological Disorders* **4**, 183-197. (doi:<http://dx.doi.org/10.2174/1568006043336302>).
- [53] Association, G.P. 1963 Physical Properties of Glycerine and Its Solutions. (Glycerine Producers' Association).

Figure Captions

Figure 1: Schematic diagram of the element scan sequence for interleaved imaging and the resulting image pair. The time taken to scan a single line, τ , is a function of the scanner (transducer response t_r), the speed of sound c , and the image depth d . The inter-frame time can be any odd multiple, m , of τ . The value $m=5$ is shown here.

Figure 2: Schematic diagram showing the motion of a single scatterer during the formation of an ultrasound image. The ultrasound beam is swept from left to right. The true spatial position of the scatterer at the start of forming the image is $P(x,y)$, where x and y are the spatial coordinates. The position of the scatterer in the image, $P'(x',y')$, is the position where the moving scatterer meets the sweeping ultrasound beam. V_x and V_y are the true velocity components of the scatterer. V'_x and V'_y are the velocity components of the scatterer estimated directly from the PIV algorithm. V_s is the sweep speed of the ultrasound beam.

Figure 3: Straight tube phantom results for flow of glycerol-water at a pulse rate of 60 bpm and maximum velocity around 1 m/s (#a in Table 1). The interleaved method was used with $\Delta t = 31\tau$ and image depth of 2 cm. A-D) Velocity vector plots at selected phases of the pulse cycle. The region has been cropped in the depth direction to focus on the tube, but the entire 38 mm width of the image is shown. For clarity, only every 8th vector in the x direction and every 4th vector in the y direction, is plotted. E) Comparison of the measured velocities with those measured by Doppler ultrasound. The standard greyscale colour map is used for the Doppler spectrum with the brightness correlated to the intensity of the Doppler signal. A rainbow colour map is used for the UIV spectrum with red representing the largest number of UIV vectors, blue representing the lowest numbers of vectors, and velocities for which there were no vectors have no colour. The sample volumes for each method are also shown. F) Comparison of the measured flow rate with that measured using a transit-time flow meter. Flow meter results are mean \pm standard deviation. G) Velocity profiles at every 2nd phase throughout the pulse cycle, obtained by averaging the velocity along the tube. Colours indicate the phase starting from blue at $t=0$. Black lines are the Womersley profiles.

Figure 4: The influence of Δt on velocity measurement for the flow shown in Fig 3 (#a in Table 1). Comparison of the measured velocities with those measured by Doppler ultrasound with (A) interleaved imaging $\Delta t=15\tau$, (B) interleaved imaging $\Delta t=95\tau$ and (C) conventional imaging $\Delta t=128\tau$. D) Comparison of the flow rate measured with different values of Δt . Comparison of velocity profiles (E) at peak velocity and (F) during deceleration.

Figure 5: Straight tube phantom results for flow of glycerol-water at a pulse rate of 200 bpm and maximum velocity around 1 m/s (#b in Table 1). Comparison of the measured velocities with those measured by Doppler ultrasound with (A) interleaved imaging $\Delta t=7\tau$, (B) interleaved imaging $\Delta t=47\tau$ and (C) conventional imaging $\Delta t=128\tau$. (D) Comparison of the flow rate measured with different values of Δt . Flow meter results are mean \pm standard deviation.

Figure 6: Straight tube phantom results for flow of glycerol-water at a pulse rate of 200 bpm and maximum velocity around 1.6 m/s (#c in Table 1). Comparison of the measured velocities with those measured by Doppler ultrasound with (A) interleaved imaging $\Delta t=7\tau$, (B) interleaved imaging $\Delta t=31\tau$ and (C) conventional imaging $\Delta t=128\tau$. (D) Comparison of the flow rate measured with different values of Δt . Flow meter results are mean \pm standard deviation. (E) Comparison of velocity profiles at peak velocity.

Figure 7 (A-D): Straight tube phantom results for flow of water at a pulse rate of 60 bpm and maximum velocity around 1.6 m/s (#e in Table 1). Comparison of the measured velocities with those measured by Doppler ultrasound with (A) interleaved imaging $\Delta t=7 \tau$, (B) interleaved imaging $\Delta t=31 \tau$ and (C) conventional imaging $\Delta t=128 \tau$. (D) Comparison of the flow rate measured with different values of Δt . (E) Results with the maximum velocity reduced to around 1 m/s (#d in Table 1). Comparison of the flow rate measured with different values of Δt . Flow meter results are mean \pm standard deviation. This can be compared with Fig 4D, which shows data for a similar flow waveform but with water-glycerol.

Figure 8: Straight tube phantom results for flow of water at a pulse rate of 60 bpm and maximum velocity around 1.6 m/s, showing the influence of image depth on flow rate measurement (#e in Table 1). Flow meter results are mean \pm standard deviation.

Figure 9: Velocity measurements in the rabbit aorta. (A) Velocity vector map for the whole field of view (only every 4th vector is plotted). (B) Comparison of the velocity in the selected region with that measured by Doppler ultrasound. (C-E) Velocity vector maps at selected phases of the cardiac cycle focusing only on the relevant area of the image (only every 4th vector in the x direction and every 2nd vector in the y direction is plotted). The aorta goes from bottom right to top left of the field of view.

Figure 10: Velocity correction due to ultrasound beam sweep. This example is for the flow shown in Fig 5 (#b in Table 1), looking at the velocity on the axis of the tube, at a location 2.3 cm along the 3.8 cm transducer. (A) Velocity measurements without correction, with correction only for the steady velocity, and with correction also for acceleration. (B) Corrections to the velocity associated with the steady velocity and acceleration. (C) Influence of the corrections on the flow rate measured at this longitudinal position.

Figures

Figure 1

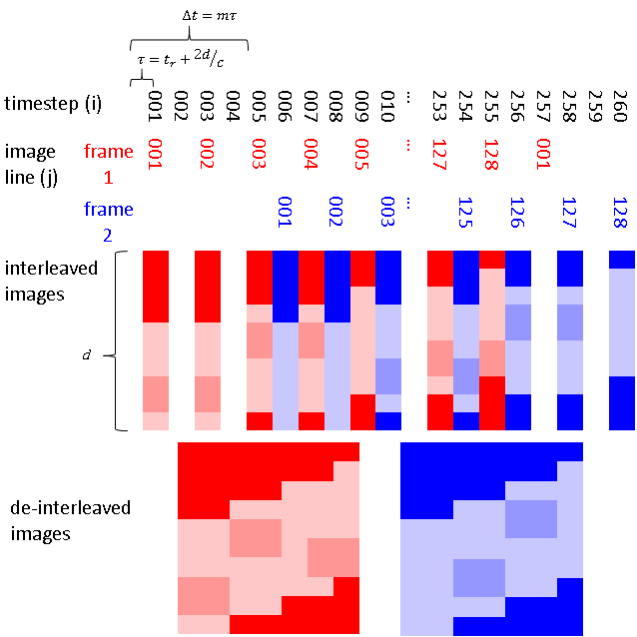


Figure 2

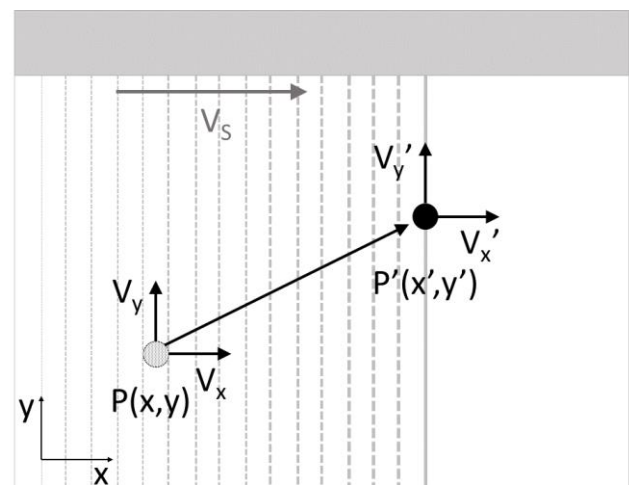


Figure 3

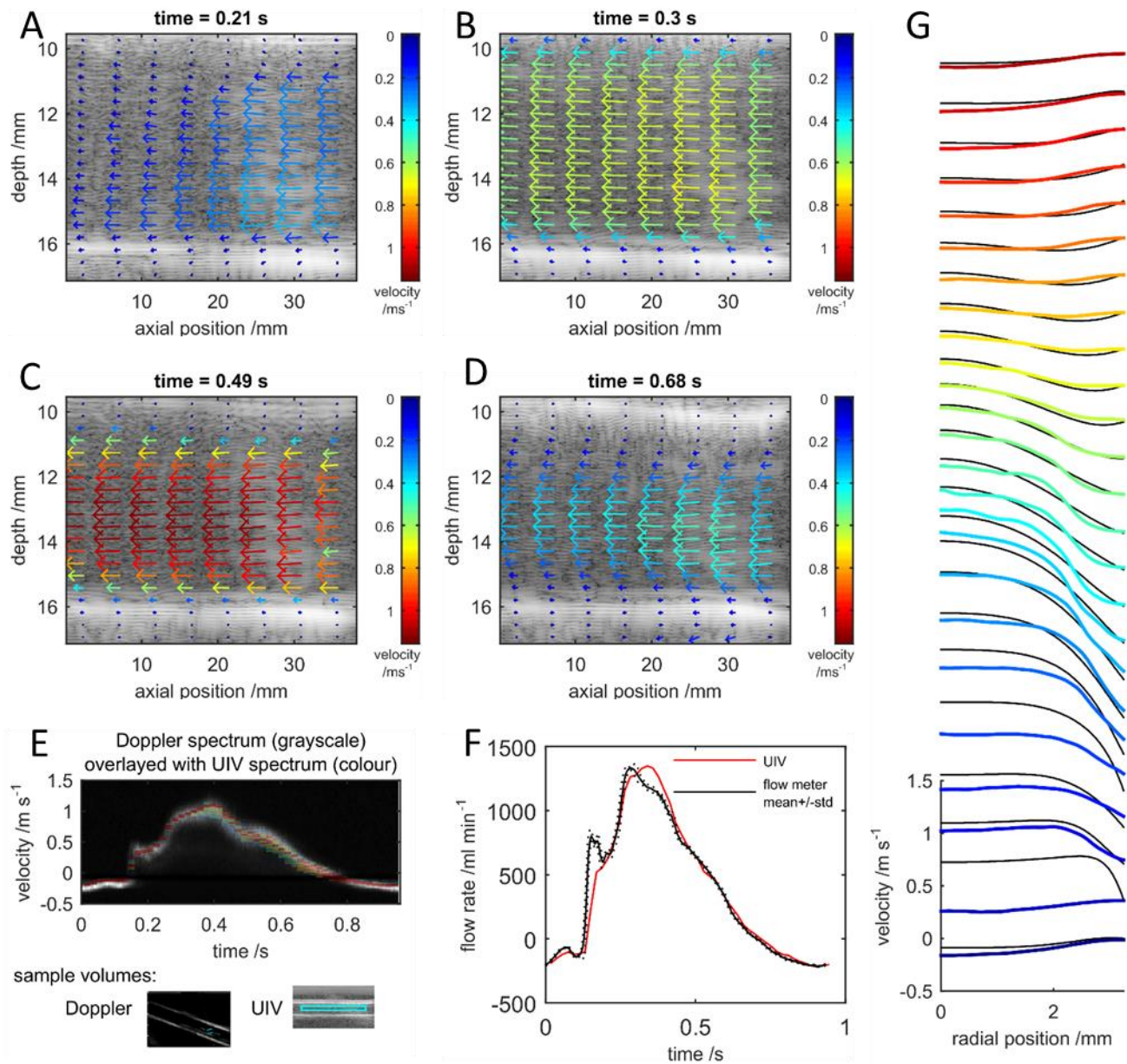


Figure 4

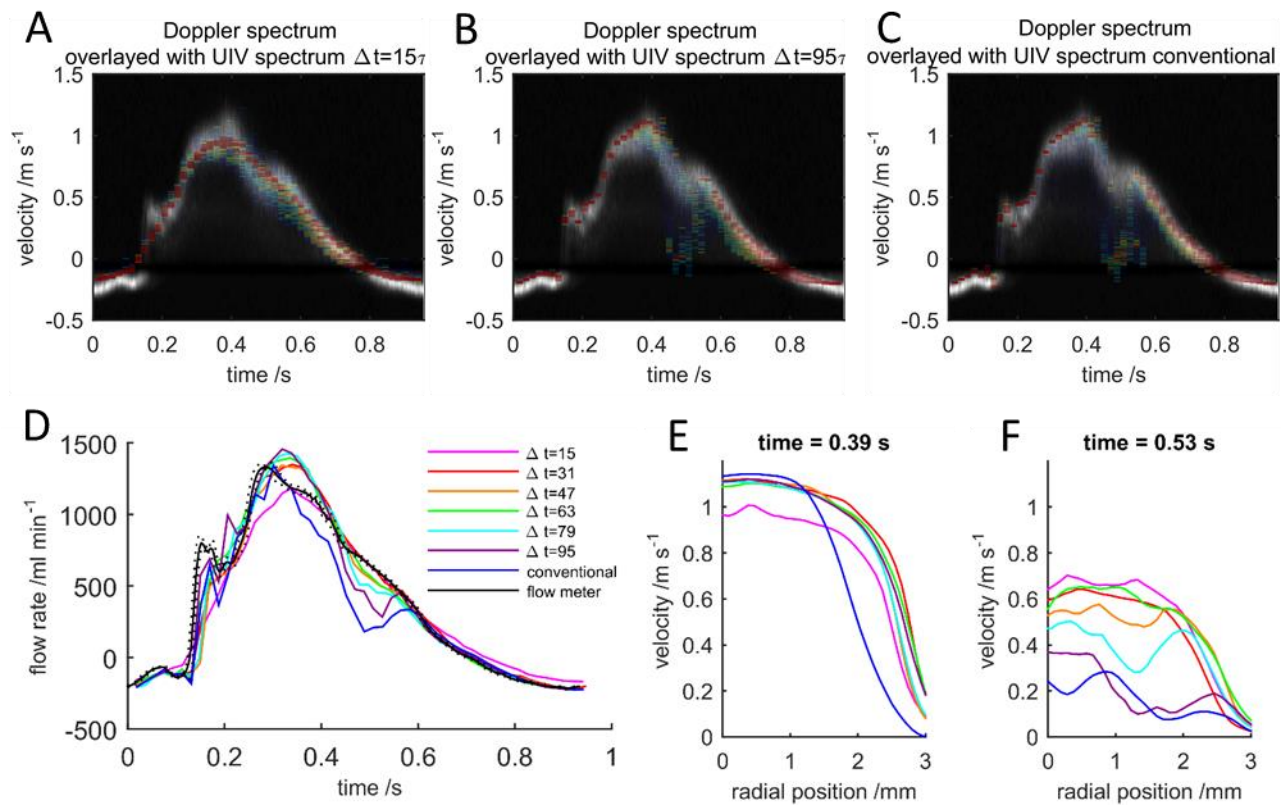


Figure 5

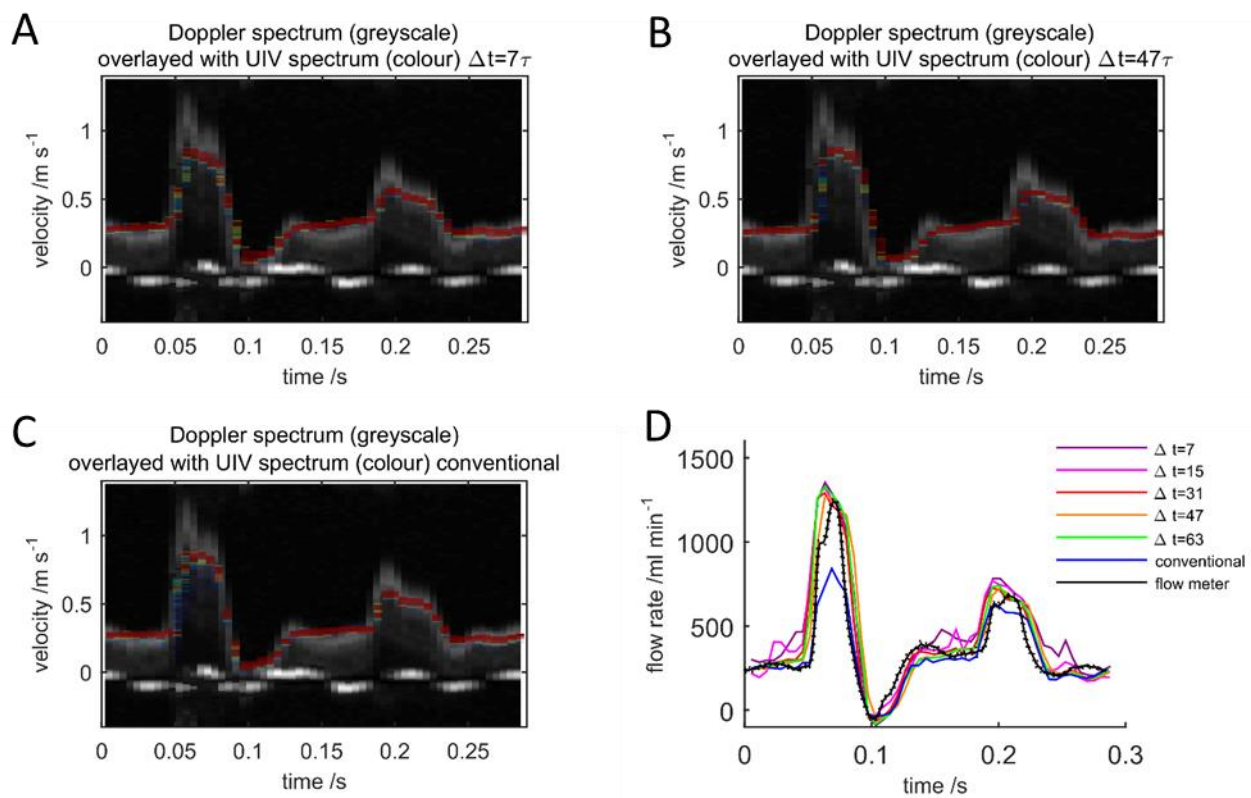


Figure 6

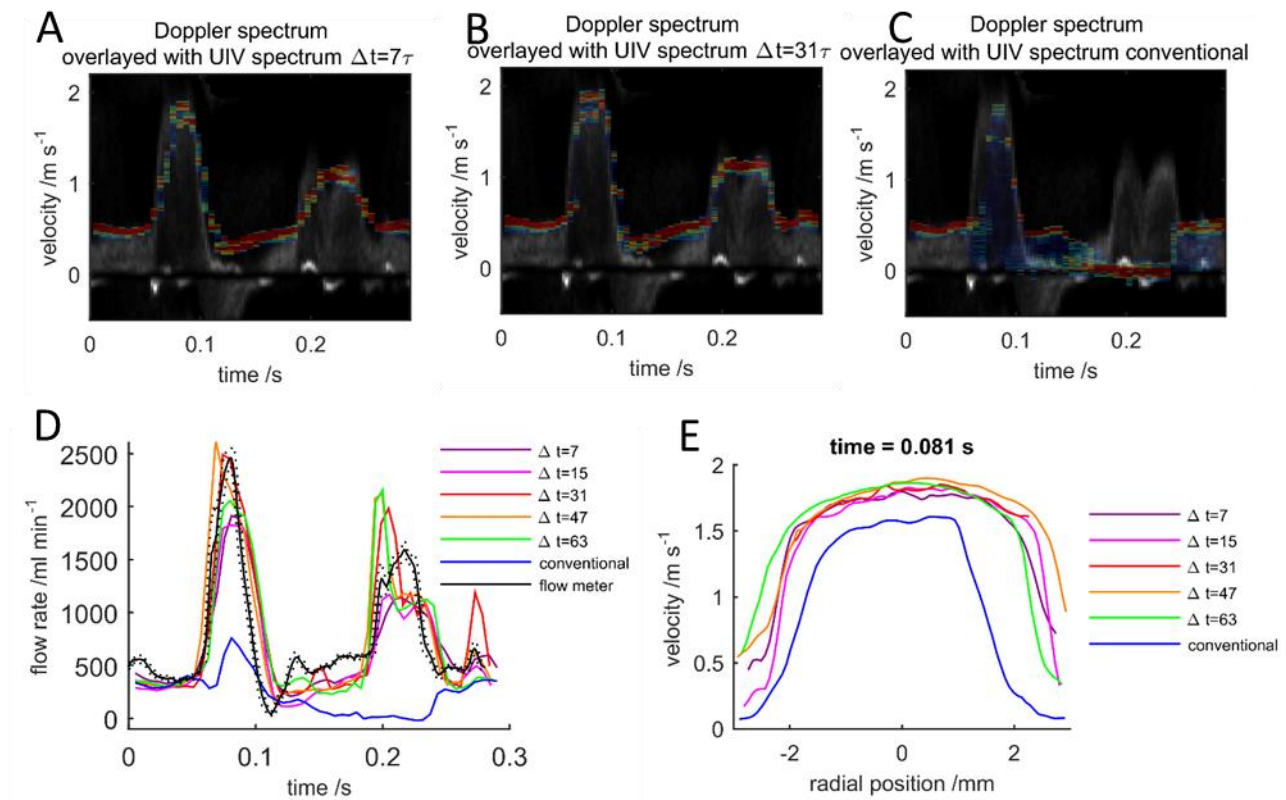


Figure 7

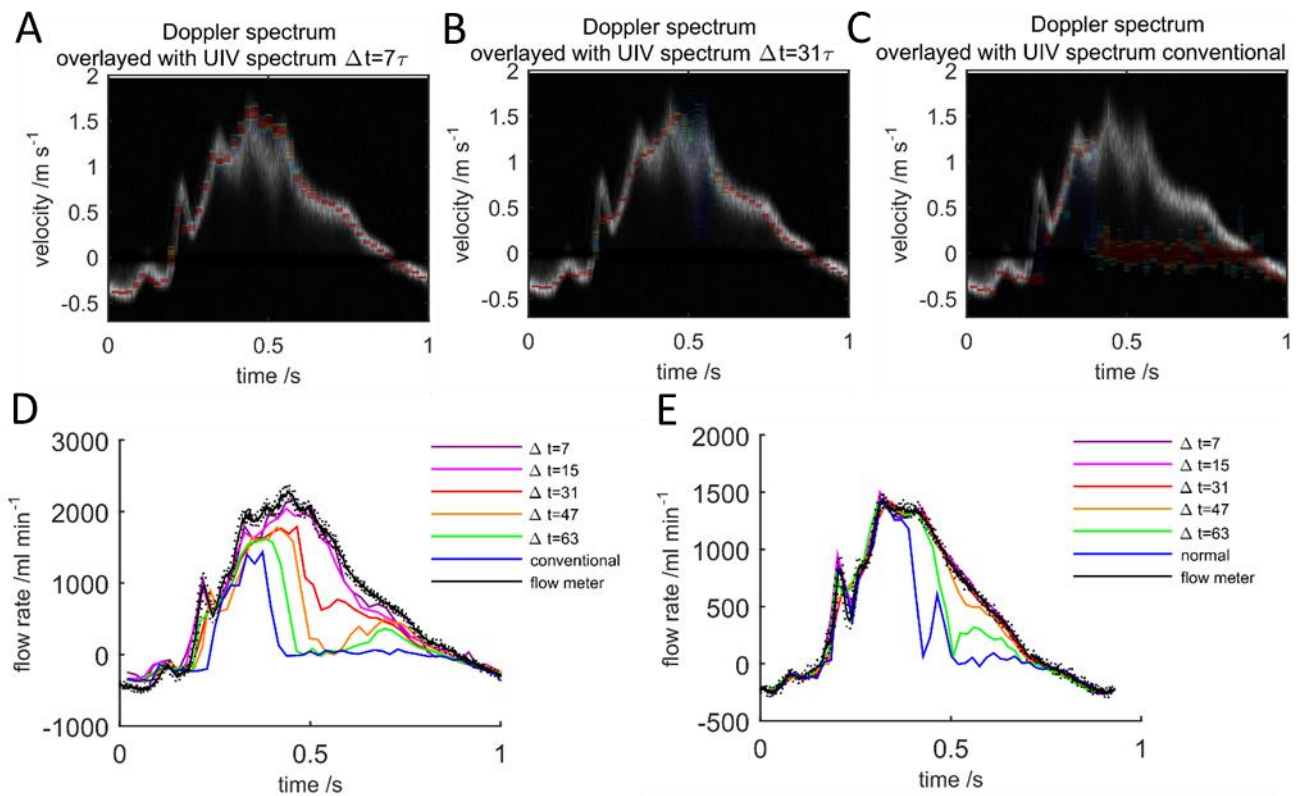


Figure 8

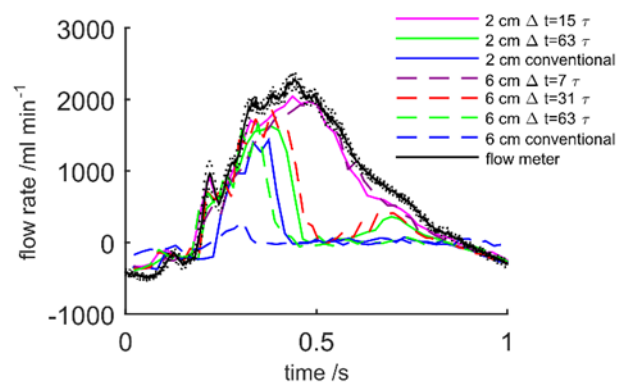


Figure 9

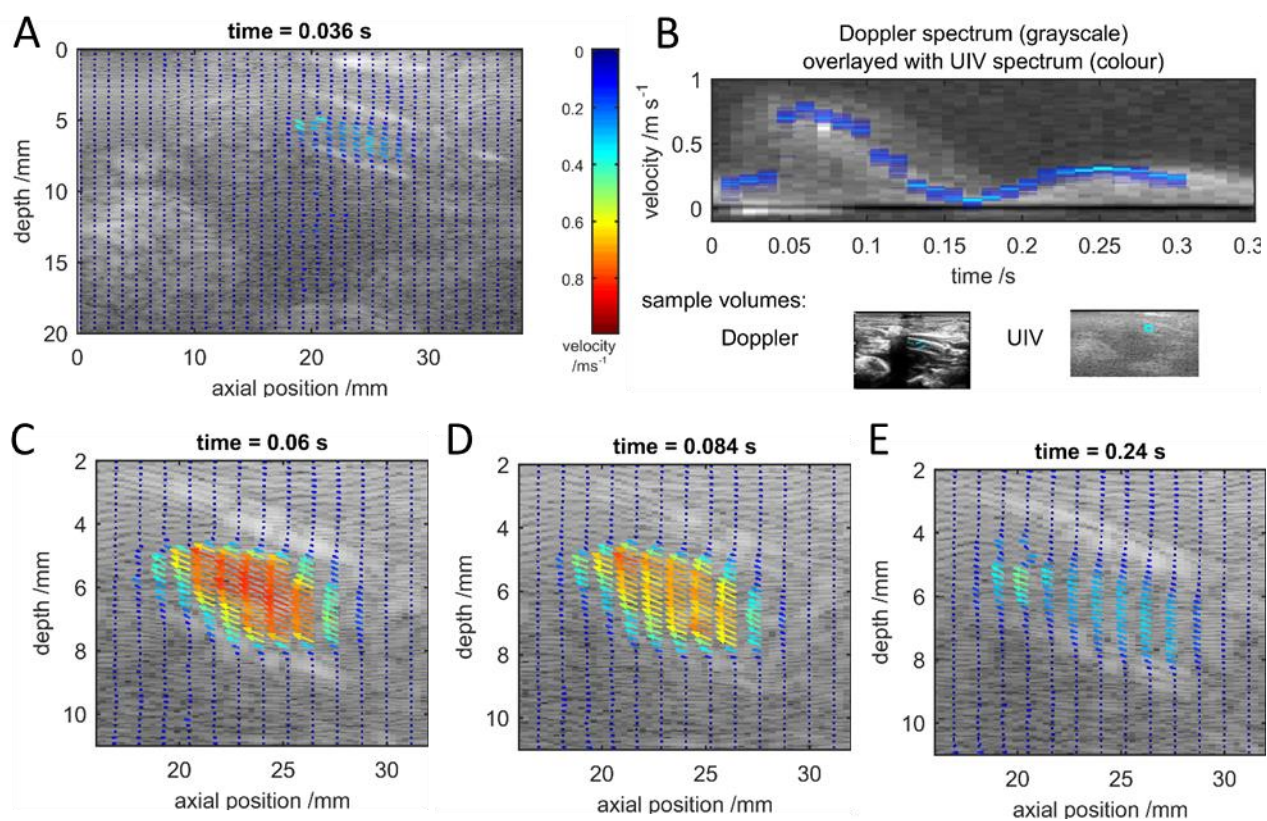


Figure 10

